

CLAIMS:

2/26 53  
1. A substantially pure nucleic acid molecule,  
comprising a sequence of nucleotides encoding an alpha  
subunit or a beta subunit of a human neuronal nicotinic  
5 acetylcholine receptor.

2/26 54 53  
2. The molecule of claim 1, wherein the  
subunit is an alpha2, alpha3 subunit or beta2 subunit.

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3. A substantially pure subunit of the human  
neuronal nicotinic acetylcholine receptor encoded by the  
10 molecule of claim 1.

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4. A substantially pure subunit of the human  
neuronal nicotinic acetylcholine receptor encoded by the  
molecule of claim 2.

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5. A nucleic acid molecule that includes a  
15 sequence of nucleotides that encodes an alpha or beta  
subunit of a human neuronal nicotinic acetylcholine  
receptor and hybridizes under conditions of high  
stringency to a sequence of nucleotides encoding a  
subunit of claim 2.

2/26 58 54  
6. The nucleic acid molecule of claim 2,  
wherein the sequence of nucleotides is selected from a  
sequence of nucleotides encoding the alpha2 subunit and  
having the restriction map of the DNA encoding the human  
alpha2 subunit set forth in Figure 1, a sequence of  
25 nucleotides encoding the alpha3 subunit and having the  
restriction map of the DNA encoding the human alpha3  
subunit set forth in Figure 2, or a sequence of  
nucleotides encoding the beta2 subunit and having the  
restriction map of the DNA encoding the human beta2  
30 subunit set forth in Figure 3.

2/26

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7. Isolated cells containing any one or more of the molecules of claim 1.

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8. Isolated cells containing one or more of the molecules of claim 2.

Sub  
E2

5

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9. The cells of claim 7 that express a nicotinic acetylcholine receptor that contains one or more subunits encoded by said nucleic acid molecule(s).

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C

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10. The cells of claim 7, wherein said cells are eukaryotic cells.

a  
cont

10 sub  
74

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11. The cells of claim 7, wherein said cells are bacterial cells, mammalian cells, yeast cells or amphibian oocytes.

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12. The cells of claim 9, wherein said cells additionally contain a reporter gene expression construct; and the reporter gene expression construct comprises:

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a transcriptional control element, and

a reporter gene encoding a transcription and/or translational product;

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said transcription control element, in said cell, is responsive to an intracellular condition that occurs when a human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor;

25

said product can be, directly or indirectly, detected; and

the gene is in operative association with said transcriptional control element.

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13. The cells of claim 12, wherein:  
the reporter gene expression construct contains a promoter selected from the group consisting of the c-fos promoter, the vasoactive intestinal peptide gene

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promoter, the somatostatin gene promoter, the proenkephalin gene promoter, the phosphoenolpyruvate carboxykinase gene promoter and the NGFI-A gene promoter, and a reporter gene selected from the group consisting of  
5 DNA encoding chloramphenicol transferase (CAT), luciferase, alkaline phosphatase and  $\beta$ -galactosidase; and the promoter is operatively linked to the reporter gene.

*12/26* *66*  
14. The cells of claim *59* *7*, wherein said cells  
10 contain nucleic acid encoding an alpha subunit and a beta subunit of a human neuronal nicotinic acetylcholine receptor.

*a1*  
*cont*  
*sub*  
*75*  
*67*  
15. The cells of claim *66* *14*, wherein said alpha subunit of the human neuronal nicotinic acetylcholine  
15 receptor is selected from the human alpha2 subunit or the human alpha3 subunit, and said beta subunit is the human beta2 subunit.

*68*  
16. A method for screening compounds for  
activity as nicotinic acetylcholine receptor agonists or  
20 antagonists, comprising determining the effect of the compound on the neuronal nicotinic acetylcholine receptor activity in the cells of claim *67* *9* compared to the effect on control cells or to the effect in the absence of the compound, wherein:

*sub*  
*e4*  
25 the activity is assessed by detecting nicotine binding to the cells, measuring the flux of ions through the membranes of the cells, measuring transcription of a reporter gene in the cells, or the electrophysiological response of the cells; and

30 control cells do not express nicotinic acetylcholine receptors.

*69*  
17. The method of claim *68* *16*, comprising:  
comparing the difference in the amount of  
transcription of a reporter gene in the cells in the

presence of the compound with the amount of transcription in the absence of the compound or with the amount of transcription in the control cells that do not express nicotinic acetylcholine receptors, wherein:

5 the cells and the control cells contain a reporter gene expression construct;

the reporter gene expression construct contains a transcriptional control element, and a reporter gene encoding a transcription and/or translational product;

10 the transcription control element, in said cell, is responsive to an intracellular condition that occurs when a human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor;

15 the product can be, directly or indirectly, detected; and

the gene is in operative association with said transcriptional control element.

*al sub H8 cont*  
*70*  
*7126*  
20 ~~18.~~ The method of claim ~~16~~ *68*, wherein the alpha subunit is an alpha2 subunit encoded by a sequence of nucleotides having the restriction map of the DNA encoding the human alpha2 subunit set forth in Figure 1 or an alpha3 subunit encoded by a sequence of nucleotides having the restriction map of the DNA encoding the human  
25 alpha3 subunit set forth in Figure 2, and the beta subunit is encoded by a sequence of nucleotides having the restriction map of the DNA encoding the human beta2 subunit set forth in Figure 3.

*71*  
30 19. A substantially pure human neuronal nicotinic acetylcholine receptor, comprising at least one human alpha receptor subunit and at least one human beta subunit.

*sub 76*  
*72*  
20. A method for making cells having neuronal nicotinic acetylcholine receptor activity, comprising:

(a) introducing one or more nucleic acid molecules of claim 1 that encode(s) at least one alpha subunit of a neuronal nicotinic acetylcholine receptor and at least one beta subunit of a neuronal nicotinic  
5 acetylcholine receptor, eukaryotic cells;

(b) selecting cells from (a) that express the alpha or beta subunit-encoding nucleic acid or express the alpha and beta subunit-encoding nucleic acid; and

(c) detecting neuronal nicotinic acetylcholine  
10 receptor activity in the selected cells, wherein the activity is mediated by a receptor containing one or more of the alpha and beta subunits encoded by said introduced nucleic acid molecules.

Applicants respectfully submit that none of these rewritten Claims constitutes new matter.

It is respectfully requested that the Claims fee be computed on the basis of the replacement Claims 1-20 submitted herewith under PCT Article 41 upon entry of this application into the U.S. National phase.

Respectfully submitted,

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